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(54) Title: METHOD FOR DIAGNOSIS OF OVARIAN DYSGENESIS (57) Abstract A method is provided for determining a follicle stimulating hormone receptor genotype in a human patient, which method is particularly useful for diagnosing ovarian dysgenesis in affected human females. The method comprises analysis of DNA from a patient which encodes all or a portion of the receptor for follicle-stimulating hormone.		

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METHOD FOR DIAGNOSIS OF OVARIAN DYSGENESIS

FIELD OF THE INVENTION

The present invention relates to methods for the detection of hereditary ovarian dysgenesis.

BACKGROUND OF THE INVENTION

Normal gonadal function depends upon the integrity of the pituitary-gonadal axis. In females, regulatory control of the ovary is primarily accomplished by the pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). At birth, development of the ovarian follicles is normally arrested until pituitary gonadotropin stimulation at puberty causes follicular maturation. Either the failure of the pituitary to secrete sufficient amounts of FSH and/or LH or the failure of the ovary to respond to gonadotropin stimulation results in hypogonadism. In females, such a condition may be characterized by the development of anatomically normal internal and external genitalia, variably developed secondary sexual characteristics, and amenorrhea. Failure of the ovaries to respond appropriately to FSH and/or LH stimulation results in poorly-developed (streak) ovaries and increased circulating levels of the hormone(s) to which the ovary is not responding. Such a condition is referred to as ovarian dysgenesis. That disease is one of a group of diseases characterized by hypogonadism which are often referred to generally as hypergonadotropic hypogonadisms. Many such diseases are due to mutations in the sex chromosomes. However, ovarian dysgenesis in females with an XX karyotype is rare and may be due to an autosomal recessive mutation. Simpson, *et al.*, *Birth Defects: Original Article Series*, 7:215-228 (1971); Aittomäki, *Am. J. Genet.*, 54:844-851 (1994).

Also of interest to the present invention are the receptors for FSH and LH. Each of those receptors is a member of a family of receptors which are coupled to GTP-binding proteins (G-proteins) in order to effect intracellular signaling. Other members of this family include certain adrenergic receptors, muscarinic cholinergic receptors, vasopressin receptors, and angiotensin receptors. Most receptors in this family comprise three distinct domains, an extracellular domain, a

thereby generating amplified DNA; exposing the amplified DNA to a restriction endonuclease selected from the group consisting of *BsmI* and restriction endonucleases having a recognition site which overlaps that of *BsmI*, under conditions wherein the restriction endonuclease specifically cleaves DNA at its recognition site; detecting polynucleotide restriction fragments of the amplified DNA; and diagnosing ovarian dysgenesis from the restriction fragments, wherein ovarian dysgenesis is correlated with a C to T mutation in codon 189 of the DNA encoding a receptor for follicle-stimulating hormone, the mutation eliminating a recognition site of the restriction endonuclease. The enzyme *BsmI* recognizes the sequence 5'-NG'CATTC-3' and cleaves between the indicated G and C. Restriction endonucleases that recognize sequences which overlap the recognition site of *BsmI*, such as *SacIII* or *SsrIII*, may also be used. *SacIII* and *SsrIII* recognize sequences internal to a *BsmI* site.

The portion of nucleic acids subjected to amplification need not include the entire coding sequence for a follicle-stimulating hormone receptor. For example, the portion of nucleic acids amplified in the method of the invention may include only exon 7, or only a portion of exon 7. Depending upon the particular portion of nucleic acid amplified in the method of the invention, and the particular restriction enzyme used, the number and/or size of restriction fragments may vary. For example, using nucleic acids comprising exon 7 of a follicle-stimulating hormone receptor (*fshr*) allele and *BsmI*, a diagnosis of ovarian dysgenesis is made if the amplified nucleic acid remained uncut when exposed to the restriction endonuclease (*i.e.*, a single fragment comprising the uncut amplified nucleic acid is observed).

In another preferred embodiment, the invention provides a method for diagnosing ovarian dysgenesis in a female patient suspected of having ovarian dysgenesis, comprising the steps of obtaining a cell sample from the female patient; isolating nucleic acid from the cell sample; amplifying a portion of the isolated nucleic acid comprising exon 7 of a follicle-stimulating hormone receptor gene, thereby generating amplified DNA; sequencing the amplified DNA; comparing the sequence of the amplified DNA to SEQ ID NO:1; and diagnosing ovarian dysgenesis by the presence of one or more sequence differences between the sequence

of the amplified DNA and SEQ ID NO:1. The sequence differences, for example, may involve a single nucleotide difference at position 640 of SEQ ID NO:1 (e.g., a thymidylate in the amplified DNA corresponding to the cytidylate at position 640 of SEQ ID NO:1).

5 Another aspect of the invention is directed to a method for determining a follicle-stimulating hormone receptor (*fshr*) genotype in a human patient, which comprises the steps of providing a biological sample comprising nucleic acid from the patient, the nucleic acid including the patient's *fshr* alleles; analyzing the nucleic acid for the presence of a mutation or mutations in codon 189 of the *fshr*
10 alleles; and determining an *fshr* genotype from the analyzing step, wherein the presence of a mutation in codon 189 of a *fshr* allele is correlated with an ovarian dysgenesis genotype. The method is useful for screening both male and female patients for determining an *fshr* genotype, for predicting/diagnosing disease states (e.g., ovarian dysgenesis in a female patient), and for genetic counseling purposes
15 (e.g., for couples intending to have children). The biological sample may be any sample from the patient containing nucleic acid suitable for analysis (e.g., tissue and fluid samples); a cell sample is a preferred biological sample. Further, the analyzing step of the invention embraces a variety of analytical techniques including sequencing all, or a portion, of a nucleic acid (which may be DNA or RNA)
20 comprising codon 189 of a *fshr* allele. By way of further illustration, the analyzing step of the invention includes restriction endonuclease analyses, wherein a nucleic acid is exposed to a restriction endonuclease having a recognition site that includes codon 189 of a wild type *fshr* allele, under conditions wherein the restriction endonuclease specifically cleaves DNA at its recognition site; and detecting
25 the nucleic acid or polynucleotide restriction fragments thereof resulting from the exposing step. Restriction endonucleases for use in this embodiment of the invention include *BsmI*, restriction endonucleases recognizing sites that overlap a *BsmI* site, restriction endonucleases recognizing the same cleavage sites as *BsmI*, and isoschizomers of *BsmI*.

30 In another aspect of the invention, a method for screening for an ovarian dysgenesis genotype in a patient comprises the steps of providing a biolo-

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gical sample comprising nucleic acid from the patient, the nucleic acid including the patient's follicle-stimulating hormone receptor (*fshr*) alleles; amplifying a portion of the nucleic acid to generate amplified DNA, the portion including codon 189 of the *fshr* alleles; exposing the amplified DNA to a restriction endonuclease selected from the group consisting of *BsmI* restriction endonuclease and restriction endonucleases having a recognition site which overlaps a *BsmI* recognition site, under conditions wherein the restriction endonuclease specifically cleaves DNA at the recognition site of the restriction endonuclease; thereafter detecting said amplified DNA or restriction fragments thereof; comparing the amplified DNA or fragments thereof to control nucleic acid of a human subject free of an ovarian dysgenesis genotype, wherein said control nucleic acid has been amplified, exposed, and detected as described above; and screening for an ovarian dysgenesis from the comparison, wherein an ovarian dysgenesis genotype in the patient is correlated with a different number of detected amplified DNA or restriction fragments thereof from the patient's amplified DNA than from the control amplified DNA. In screening for ovarian dysgenesis genotypes, for example, a homozygous ovarian dysgenesis genotype may be correlated with fewer detected restriction fragments from the patient's amplified DNA than from the control amplified DNA. Consistently, the screening may correlate a heterozygous ovarian dysgenesis genotype with a greater number of detected amplified DNA or restriction fragments thereof from the patient's amplified DNA than from the control DNA.

Yet another aspect of the invention is a method comprising the steps of obtaining a cell sample from a female patient; isolating DNA from the cell sample; amplifying a portion of the DNA encoding exon 7 of the follicle-stimulating hormone receptor; exposing the amplified DNA to *BsmI* or another restriction endonuclease which recognizes the *BsmI* site; and diagnosing ovarian dysgenesis as the presence of a single fragment upon digestion.

Additional aspects of the invention will become apparent upon consideration of the following detailed description thereof.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of the human FSH receptor showing the multi-exon structure of the gene encoding the protein; the extracellular (Ec), transmembrane (Tm), and intracellular (Ic) domains of the receptor; and the
5 Ala to Val and Asn to Ser transitions (boxed) described herein.

Figure 2 shows a pedigree of families used in studies of ovarian dysgenesis inheritance patterns.

Figure 3 is a map of microsatellite markers on human chromosome
2.

10 Figure 4 depicts the nucleotide sequence, and deduced amino acid sequence, of the FSH receptor (SEQ ID NO:1) gene, showing *BsmI* sites in bold.

Figure 5 is a graph showing results of FSH-induced cAMP production by MSC01 cells transfected with wild-type or mutant FSH receptor-encoding DNA.

15 Figure 6 is a graph showing Scatchard analysis of FSH binding to MSC-1 cells transfected with wild-type (circles) or mutated (triangles) FSH receptor-encoding DNA.

DETAILED DESCRIPTION OF THE INVENTION

20 The present invention provides methods for the diagnosis of diseases characterized by hypergonadotropic hypogonadisms such as ovarian dysfunction. In particular, methods of the invention are useful in the diagnosis of ovarian dysgenesis in females. Diagnostic methods according to the invention may be used at any time in the life of the patient, even prior to the onset of symptoms, such as the
25 failure of normal onset of menstruation at puberty. Methods of the invention are primarily based upon the discovery that a mutation in the coding sequence of the gene for the follicle-stimulating hormone receptor accounts for the inability of FSH to generate signal transduction at its receptor.

In the normal genotype for the follicle-stimulating hormone receptor,
30 four *BsmI* restriction sites (5'-NGCATTC-3') exist, as shown in Figure 4 (SEQ ID NO:1). In individuals having ovarian dysgenesis, the FSH receptor gene has been observed to have a mutation at position 640 (SEQ ID NO:1), such that a thymine

is substituted for a cytosine, resulting in the sequence, 5'-NGTATTC-3'. This substitution eliminates the *BsmI* site spanning nucleotides 638-644 in the normal (wild type) *fshr* gene. Thus, digestion of DNA encoding a wild-type FSH receptor with *BsmI* normally produces five fragments (due to cleavage at all four *BsmI* sites in a linear DNA). However, the thymine-to-cytosine mutation at position 640 results in a mutated DNA that produces only four restriction fragments upon *BsmI* digestion. As shown below, mutation at position 640 is uniquely associated with ovarian dysgenesis; whereas no mutation at any of the other three *BsmI* sites in the FSH receptor DNA has been found in patients suffering from that disease. Accordingly, *BsmI* digestion and subsequent observation of the products produced is useful as a diagnostic method or a screening method for ovarian dysgenesis. As detailed below, position 640 in SEQ ID NO:1 falls within exon 7 of the *fshr* gene. In one embodiment of the invention, the region of exon 7, or a portion thereof, of the FSH receptor coding sequence may also be isolated and exposed to *BsmI*.

Exon 7 contains a unique *BsmI* site which, if mutated, will produce no *BsmI* digestion products, thereby enabling diagnosis of ovarian dysgenesis.

The gene encoding the FSH receptor has been mapped to human chromosome 2p16-21. Rousseau-Merck, *et al.*, *Genomics*, 15:222-224 (1993). The FSH receptor coding sequence is shown in SEQ ID NO:1 and in Figure 4 and is available in the Genbank database as Accession No. S59900. Figure 1 shows a schematic of the FSH receptor showing the regions encoded by the 10 exons.

The following examples illustrate preferred means for detecting FSH receptor genotypes generally and for diagnosis of ovarian dysgenesis in human females.

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EXAMPLE 1

Venous blood samples were obtained from 37 individuals, each belonging to one of 6 families having at least two females with symptoms of ovarian dysgenesis. Total DNA was isolated from fresh white blood cells or lymphoblastoid cell lines established from the samples. The method of Chomczynski, *et al.*, *Anal. Biochem.*, 162:156-159 (1987), incorporated by reference herein, was

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used to isolate RNA from the samples. A pedigree showing inheritance patterns in individuals used to isolate the ovarian dysgenesis locus is shown in Figure 2.

Linkage was investigated using Généthon microsatellite markers as reported in Weissenbach, *et al.*, *Nature*, 359:794-801 (1992), incorporated by reference herein. A linkage map for chromosome 2 is provided in Figure 3. Forty-seven markers were chosen at approximately 20 centimorgan (cM) intervals. Pairwise linkage analyses were carried out using the MLINK subprogram of the LINKAGE program package reported in Lathrop, *et al.*, *Proc. Nat'l. Acad. Sci. (USA)*, 81:3443-3446 (1984), incorporated herein by reference. The disease allele frequency was set to 0.01 and lod scores were computed under a model of equilibrium between the disease locus and each marker locus, assuming a recessive mode of inheritance with full penetrance in females. A lod score is defined as the \log_{10} of the ratio of the probability that the data obtained would have arisen from unlinked loci and represents a criterion for assigning a given restriction fragment length polymorphism to a particular linkage group. Linkage was confirmed using the G2113A transition located in exon 10 of the FSH receptor gene as a polymorphic marker (see below). Amplification was accomplished using PCR according to the method of Sankila, *et al.*, *Hum. Mol. Genet.*, 4:93-98 (1995), incorporated by reference herein.

Preliminary evidence of linkage of the ovarian dysgenesis phenotype was noted for markers D2S134 and D2S177 on chromosome 2. Linkage was confirmed using markers D2S119 and D2S123, each of which is located between D2S134 and D2S177. Markers D2S119 and D2S123 define an approximately 12 cM region of chromosome 2. That region was further studied using markers D2S391, D2S288, CA21 and CA7, each of which is located in the 12 cM region defined above, and linkage of the ovarian dysgenesis locus to this region of chromosome 2 was confirmed.

Pairwise linkage analyses produced 46 potentially informative meioses, of which 30 were from affected females. The lod scores obtained from those meioses revealed no recombinations between the markers and the ovarian dysgenesis locus as evidenced by phenotype. The highest lod score, $Z_{\max} = 4.71$, was obtained for marker D2S391, while two other marker loci, D2S288 and CA21,

provided lod scores exceeding the limit of proven linkage ($Z \geq 3.0$). The region in which linkage of the ovarian dysgenesis phenotype exists was, therefore, isolated to the 2p region of chromosome 2 based upon the above linkage analysis. That region of chromosome 2 is the region which contains the genes encoding the receptors for follicle-stimulating hormone and luteinizing hormone. Thus, it was hypothesized that a nonlethal mutation in one of these two genes was responsible for ovarian dysgenesis. On the basis that an LH receptor mutation would also cause severe hypogonadism in males and no males were affected in the pedigrees producing affected females, the LH receptor gene was considered unlikely as a site of mutation causing ovarian dysgenesis. Accordingly, the gene encoding the FSH receptor was investigated as a source of the ovarian dysgenesis phenotype.

EXAMPLE 2

As shown in Figure 1, the FSH receptor gene comprises 10 exons. As recited above, the polynucleotide sequence of the entire *fshr* coding region can be obtained from Genbank under Accession Number S59900. The polynucleotide sequence of each one of the ten exons of *fshr* has also been deposited under individual accession numbers in the Genbank database. A comparison of the sequences of the individual exons to the coding region of the entire coding region presented in SEQ ID NO:1 reveals that a few terminal nucleotides from each exon are lost in the process of forming a mature mRNA during expression. The correlation between the sequences of the individual exons and the sequence presented in SEQ ID NO:1 follows. Nucleotides 1-158 of exon 1 (Acc. No. X91738) are found at positions 70-227 of SEQ ID NO:1; nucleotides 7-77 of exon 2 (Acc. No. X91739) span positions 228-298 of SEQ ID NO:1; nucleotides 6-80 of exon 3 (Acc. No. X91740) are found at positions 299-373 of SEQ ID NO:1; nucleotides 6-82 of exon 4 (Acc. No. X91741) span positions 374-450 of SEQ ID NO:1; nucleotides 8-77 of exon 5 (Acc. No. X91742) are found at positions 451-520 of SEQ ID NO:1; nucleotides 6-83 of exon 6 (Acc. No. X91743) span positions 521-598 of SEQ ID NO:1; nucleotides 6-75 of exon 7 (Acc. No. X91744) are found at positions 599-668 of SEQ ID NO:1; nucleotides 7-80 of exon 8 (Acc. No. X91745) span positions 669-742 of SEQ ID NO:1; nucleotides 6-191 of exon 9 (Acc. No.

X91746) are found at positions 743-928 of SEQ ID NO:1; and nucleotides 102-1352 of exon 10 (Acc. No. S73526) span positions 929-2179 of SEQ ID NO:1.

A systematic analysis was performed to determine the change or changes in that gene which are responsible for expression of the ovarian dysgenesis phenotype.

The large terminal exon of the FSH receptor was screened for mutations using denaturing gradient gel electrophoresis. Exon 10 was amplified with GC clamped primers in four different PCR reactions with overlapping products covering the entire transmembrane and intracellular domains. Pairs of GC clamped primers used in PCR were primer 10f: 5'-CGCCCGCCGCGCCCCGCGCCCGCCCGCCCGCCCCGCGCCCGGACTTATGCAATGAAGTGGTTG-3' (forward, SEQ ID NO:2), 10r: 5'-GTGAAAAGCCAGCAGCATC-3' (reverse, SEQ ID NO:3); primer 11f: 5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCCGCCCCGCCCGATTGACTGGCAAAGTGGGG-3' (forward, SEQ ID NO:4), 11r: 5'-AGAGGAGGACACGATGTTGG-3' (reverse, SEQ ID NO:5); primer 12f: 5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCCGCCCCGCCCGGGCTGCTATATCCACATCTACC-3' (forward, SEQ ID NO:6), 12r: 5'-CAGAACCAGCAGAATCTTTGC-3' (reverse, SEQ ID NO:7); and primer 13f: 5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCCGCCCCGCCCGCTTTCTTTGCATTTCTGCC-3' (forward, SEQ ID NO:8), 13r 5'-CAAAGGCAAGGACTGAATTATC-3' (reverse, SEQ ID NO:9). Each pair of primers was optimized for each fragment with the MELT 87 program described in Lerman, *et al.*, *Methods in Enzymology* (Wu, *et al.*, eds. 1989), incorporated by reference herein. For each PCR run, samples were heated to 94°C for 4 minutes and put through 34 cycles. For each cycle, denaturation was conducted for 1 minute at 94°C, annealing was conducted for 1 minute at 57°C, 58°C, 57°C, and 54°C for primer pairs 10, 11, 12, and 13, respectively, and extension was for 30 seconds at 72°C.

A 7% polyacrylamide gel was designed for each fragment with a linear concentration gradient of formamide. Gels were run in an aquarium at a stable temperature of about 60°C for 16-20 hours in a denaturing gel electrophoresis system, model DGGE2000 (C.B.S. Scientific Co.). Denaturing gradient gel electrophoresis procedures are generally known in the art and are available, for

example, in Ausubel, *et al.* (eds.) *Current Protocols in Molecular Biology*, 2.12, *et seq.* (1987), incorporated by reference herein.

A sequence polymorphism was detected in the terminal region of exon 10. A 326 bp fragment beginning at nucleotide 1892 of the FSH receptor coding sequence was amplified using primers 14f: 5'-AGCAAAGATTCTGCTGG-TTC-3' (forward, SEQ ID NO:10) and 14r: 5'-CAAAGGCAAGGACTGAAT-TATC-3' (reverse, SEQ ID NO:9). The amplified PCR product was sequenced using the dideoxy chain termination method as reported in *Molecular Cloning: A Laboratory Manual* (Sambrook, *et al.*, eds 1989), incorporated by reference herein. A G-to-A transition was observed in the sequenced product at nucleotide position 2113 (G2113A transition). Position 2113 of SEQ ID NO:1 is in the region encoding the intracellular domain of the FSH receptor and predicts a change from Ser to Asn at amino acid position 680 of the corresponding protein structure. The G2113A transition abolishes a *Bsr*I site, thus enabling *Bsr*I to be used as a screen for the two alleles. The polymorphism at that site was designated FSHR1.

The G2113A transition observed in exon 10 of the FSH receptor coding sequence had previously been identified as a difference between the ovarian and testicular forms of the gene encoding the FSH receptor. Kelton, *et al.*, *Mol. Cell. Endocrinol.*, 89:141-151 (1992). Denaturing gradient gel electrophoresis analysis of samples obtained from a family having females both with and without ovarian dysgenesis showed that the allelic form of the gene at the G2113A transition had no effect on phenotype. Thus, the allelic variance at position 2113 was not considered to be disease causing. No recombinations between that locus and the disease phenotype were observed, as shown in Table 1.

TABLE 1

Marker locus	Recombination fraction, θ							90% support interval
	0.00	0.001	0.01	0.05	0.10	0.20	0.30	
<i>D2S119</i>	2.41	2.41	2.35	2.12	1.82	1.20	0.62	0-0.17
<i>D2S391</i>	4.71	4.70	4.59	4.14	3.56	2.38	1.26	0-0.09
<i>D2S288</i>	3.10	3.10	3.03	2.72	2.33	1.52	0.78	0-0.13
<i>CA21</i>	4.20	4.19	4.11	3.74	3.24	2.19	1.16	0-0.10
<i>CA7</i>	2.66	2.66	2.60	2.35	2.03	1.36	0.71	0-0.16
<i>D2S123</i>	2.68	2.67	2.62	2.39	2.08	1.44	0.79	0-0.16
<i>FSHR1</i>	2.72	2.71	2.66	2.44	2.14	1.49	0.82	0-0.16

The *FSHR1* locus was, however, used as a marker in the linkage analysis described above. Since the polymorphism in exon 10 was excluded as the source of the ovarian dysgenesis phenotype, the other exons of the FSH receptor were screened.

EXAMPLE 3

Exons 1-5 and 9 were screened by amplifying each with flanking intronic primers and sequencing the PCR product. None of those exons showed a mutation associated with the ovarian dysgenesis phenotype.

Complete sequences for the flanking introns of exons 6, 7, and 8 were not available. However, it was determined that FSH receptor-encoding mRNA could be isolated by using RNA from blood leukocytes as a template. Such a process takes advantage of so-called illegitimate transcription, whereby small amounts of mRNA encoding most tissue-specific proteins are produced by white blood cells. Due to the sensitivity of PCR and since PCR products can be directly sequenced, small amounts of RNA produced by white blood cells were used to amplify the FSH receptor coding region. RNA was isolated from white

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blood cells and exons 6-9 were amplified by reverse transcription PCR in order to identify mutations by direct sequencing of the PCR products as follows.

Total RNA was isolated from white blood cells or from lymphoblasts. Approximately 0.8 μ g RNA was used as a template for first-strand cDNA synthesis and primed for reverse transcription by 40 pmol of primer 15r: 5'-TAGTTTTGGGCTAAATGACTTAGAGGG-3' (SEQ ID NO:11), which is complementary to nucleotides 2161-2135 of SEQ ID NO:1. Approximately 1mM each of dATP, dCTP, dTTP, dGTP and 200 U M-mLv reverse transcriptase were combined in M-mLv reverse transcriptase buffer (Promega, Madison, WI) to a final volume 20 μ L. Samples were incubated at 42°C for 1 hour. Samples were then heated to 95°C for 10 minutes and a 5 μ l aliquot of the resulting cDNA product was used as a template for PCR.

The cDNA was amplified in two rounds of PCR using a nesting strategy in order to increase yield and specificity. In the first round of PCR, primer 15r (see above) and primer 16f: 5'-CCTGCTCCTGGTCTCTTTGCTG-3' (SEQ ID NO:12) were used. The reaction was heated for 2 minutes at 94°C and put through 20 cycles each comprising 1 minute denaturing at 94°C, 1 minute annealing at 58°C and 2 minutes extension at 73°C to produce a 2082 bp product. The product contained exons 6-9 and 5 μ l was used as a template for amplification of exon 7 using primer 6f: 5'-AGAAATTCTTTCGTGGGGCT-3' (forward, SEQ ID NO:13) and 6r: 5'-GTTTGCAAAGGCACAGCAAT-3' (reverse, SEQ ID NO:14). The resulting PCR product was a 357 bp fragment corresponding to nucleotides 558-914 of SEQ ID NO:1.

The 357 bp product, which included exon 7, was sequenced. Upon inspection of the resulting sequence in a number of patients (both affected and unaffected), it was noticed that all affected individuals were homozygous for a C-to-T transition at nucleotide 640 (C640T transition) of the FSH receptor sequence shown in SEQ ID NO:1. That change predicts an Ala-to-Val substitution at amino acid position 189 in the corresponding protein primary structure. The C640T transition segregated perfectly with the disease phenotype and all affected individuals were determined to be homozygous recessive for the mutation from cysteine to thymine. The mutation at position 640 abolishes a *BsmI* restriction site, thus

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enabling diagnosis of the disease by digestion of FSH receptor DNA with *BsmI*. Thus, it was determined that the C640T transition in exon 7 of the FSH receptor gene is predictive of ovarian dysgenesis in affected individuals.

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EXAMPLE 4

Digestion of genomic DNA with *BsmI* was next used in order to confirm the use of that enzyme in the diagnosis of ovarian dysgenesis.

Genomic DNA isolated from 15 affected and 22 unaffected individuals was amplified by PCR by heating for 12 minutes at 94°C followed by 30 cycles at 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes using primers 7f: 5'-GTTATTTTCAGATGGCTGAATAAG-3' (SEQ ID NO:15) and 7r: 5'-GCTCATCTAGTTGGGTTC-3' (SEQ ID NO:16). These primers were selected to amplify a 78 base pair portion of genomic DNA encompassing a portion of the intron between exons 6 and 7 and most of exon 7 of the follicle stimulating hormone receptor gene. Exon 7 of the wild type *fshr* gene contains a unique *BsmI* site at nucleotides 638-644 of SEQ ID NO: 1 which is abolished by the C640T transition observed in individuals with the ovarian dysgenesis phenotype (the three additional *BsmI* sites in the FSH receptor coding sequence shown in Fig. 4 are not in exon 7 and are not included in the above-described PCR product). Fifteen microliters of the resulting 78 bp PCR product was digested to completion with 20 IU of *BsmI* (Promega). The product of digestion was fractionated on a 10% non-denaturing polyacrylamide gel and the DNA was visualized with ethidium bromide.

In unaffected individuals, the PCR product is cleaved into 51 bp and 27 bp fragments by *BsmI*. However, in affected individuals, only a 78 bp fragment appears in the gel. Heterozygous individuals, such as the parents shown in the Family 7 pedigree in Figure 2, show all three fragments. Thus, *BsmI* digestion is probative of ovarian dysgenesis by detecting the C640T transition. The pathogenic role of the C640T transition was further confirmed in FSH receptor signaling and binding studies.

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EXAMPLE 5

Human FSH receptor-encoding DNA was obtained by reverse transcription PCR using testicular poly (A⁺) RNA as reported in Gromoll, *et al.*, *Biochem. Biophys. Res. Commun.*, 188:1077-1083 (1992), incorporated by reference herein. The resulting PCR product was subcloned into a pBluescript SR(-) vector (Stratagene Inc., La Jolla, CA). A 5' untranslated region which contained a stop codon was deleted and a Kozak translation initiation sequence was added. The resulting construct comprised 2088 bp of the FSH receptor coding region with a 5-base 5' extension and a 92-base 3' extension. The FSH receptor coding sequence with 5' and 3' extensions was excised by digestion with *Sma*I and *Kpn*I and blunt-end ligated into a blunted *Eco*RI site in the pSG5 vector (Stratagene).

A plasmid comprising the mutated receptor (*i.e.* the C640T allele) was created by site-directed mutagenesis of the wild-type cDNA. Site-directed mutagenesis was accomplished using a Clontech Transformer™ site-directed mutagenesis kit (Clontech, Palo Alto, CA) according to the manufacturer's instructions. The selection primer, 5'-GAGTGCACCATGGGCGGTGTGAAAT-3' (SEQ ID NO:17), transformed an *Nde*I site into an *Nco*I site in the vector. The mutagenesis primer, 5'-GGGATTCAAGAAATACACAACTGTGTATTCAATGGAACCC-3' (SEQ ID NO:18), accomplishes the C-to-T transition at position 640. Plasmid sequences were verified by restriction digestion and sequencing.

An MSC-1 cell line was used for transfection. That cell line was derived from a transgenic mouse Sertoli cell tumor generated by expressing the SV40 virus T-antigen under the control of the anti-muellerian hormone promoter reported in Peschon, *et al.*, *Mol. Endocrinol.*, 6:1403-1411 (1992), incorporated by reference herein. Despite their Sertoli cell origin, MSC-1 cells do not express endogenous FSH receptor. MSC-1 cells in exponential growth phase were transiently transfected with either the wild-type or mutated FSH receptor-containing plasmids described above. Transfection was accomplished in DMEM/F12 (1:1) medium using lipofection (Gibco Life Technologies, Inc.) according to the manufacturer's instruction. A mock transfection with buffer was run as a control. Transfection efficiency was maintained by cotransfection with a luciferase-expressing pCmv-luci plasmid as reported in Gossen, *et al.*, *Proc. Nat'l. Acad. Sci.*

(USA), 89:5547-5551 (1992). Transfected cells were cultured in 2 cm culture plates.

Seventy-two hours after transfection, cells were exposed to one of 2, 10, 50, 100, or 200 IU/L of recombinant human FSH (rhFSH, Org 32489, approximately 10,000 IU/mg, Organon International BV). A control (vehicle only) was also run. After 3 hours, cells and media were removed from the culture plates and divided into two equal aliquots. One of the aliquots was diluted 1:1 with 2 mM theophylline, heated for 5 minutes at 100°C, spun for 5 minutes at 1500 g; and used to measure cAMP activity as described in Harper, *et al.*, *J. Cycl. Nucleotide Res.*, 1:207-218 (1975), incorporated by reference herein.

Stimulation with rhFSH of MSC-1 cells transfected with wild-type FSH receptor DNA produced a 3-4 fold dose-dependent stimulation of cAMP. The ED₅₀ of stimulation was approximately 75 IU/L. In contrast, cells transfected with DNA comprising the C640T allele or mock-transfected with only pCmv-luci produced only negligible increases in cAMP activity, indicating that such cells are not stimulated by FSH. The results are shown in Figure 5, wherein squares denote transfections with the wild type allele, circles denote transfections with the mutant allele, and triangles represent mock transfections. Each data point in Figure 5 represents the mean of results in three identical experiments.

The foregoing results show that the ovarian dysgenesis allele (*i.e.* that with a substitution of T for C at position 640) results in the expression of a receptor which is unable to produce a signal upon stimulation by FSH.

EXAMPLE 6

MSC-1 cells which had been transfected with either mutant or wild-type FSH receptor plasmids were next used in FSH binding studies performed 48 hours after transfection. Cells were recovered and reconstituted to a concentration of 2×10^6 cells/ml in buffer. The rhFSH described above was radiolabelled with ¹²⁵I iodine using the solid-phase lactoperoxidase method of Karonen, *et al.*, *Anal. Biochem.*, 67:1-10 (1975), incorporated by reference herein, to a specific activity of 30 Ci/g and 20% specific binding of radioactivity to an excess of FSH receptor as determined according to Catt, *et al.*, *Methods in Receptor Research* (Belcher,

ed. 1976) 175-250, incorporated by reference herein. Triplicate aliquots of 100 μ l each of the cell suspension (containing approximately 200,000 cells each) were incubated in the presence of 3.13, 6.25, 12.5, 25, 50, or 100 ng of radiolabelled rhFSH in a total volume of 250 μ l. Non-specific binding was determined in the presence of 1.5 IU rhFSH. After overnight incubation at room temperature, radio-activity was measured in a gamma spectrometer.

Scatchard analysis revealed a 28-fold increase in binding of FSH to cells which had been transfected with wild-type FSH receptor-encoding DNA compared to cells transfected with DNA encoding the C640T allele. The equilibrium constant of FSH binding was $K_d = 6.7 \times 10^9$ L/mol for the wild-type receptor and 4.8×10^9 L/mol for the mutated receptor. Results are shown in Figure 6, wherein circles represent wild-type receptors and triangles represent mutant receptors. In each case specific binding was equalized to a constant amount of luciferase activity. As shown in Figure 6, specific binding was 18.2 pmol/L for the wild type and 0.63 pmol/L for the ovarian dysgenesis allele.

The foregoing results show that a mutation from C to T at position 640 of the FSH receptor coding sequence is responsible for ovarian dysgenesis in human females and that because that mutation abolishes a *BsmI* site, digestion with *BsmI* or another restriction endonuclease which recognizes the *BsmI* site is useful as a diagnostic tool for ovarian dysgenesis. It is apparent from the foregoing that other mutations may also produce the ovarian dysgenesis phenotype. Comparison of the nucleotide sequence of the FSH receptor gene in those cases with the wild-type sequence also provides a basis for diagnosis of the disease.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Helsinki University Licensing Ltd Oy
- (B) STREET: Viikinkaari 8 A
- (C) CITY: Helsinki
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- (F) POSTAL CODE: FIN-00710

(ii) TITLE OF INVENTION: Method For Diagnosis Of Ovarian Dysgenesis

(iii) NUMBER OF SEQUENCES: 18

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(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US 08/531,070
- (B) FILING DATE: 20-SEP-1995

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- (A) TELEPHONE: 358 0 648606
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2179 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 75..2159

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTGGAGCTT CTGAGATCTG TGGAGGTTTT TCTCTGCAAA TGCAGGAAGA AATCAGGTGG

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ATGGATGCAT AATT	ATG	GCC	CTG	CTC	CTG	GTC	TCT	TTG	CTG	GCA	TTC	CTG	110
	Met	Ala	Leu	Leu	Leu	Val	Ser	Leu	Leu	Ala	Phe	Leu	
	1				5					10			
AGC TTG GGC TCA GGA TGT CAT CAT CGG ATC TGT CAC TGC TCT AAC AGG	158												
Ser Leu Gly Ser Gly Cys His His Arg Ile Cys His Cys Ser Asn Arg													
	15				20					25			
GTT TTT CTC TGC CAA GAG AGC AAG GTG ACA GAG ATT CCT TCT GAC CTC	206												
Val Phe Leu Cys Gln Glu Ser Lys Val Thr Glu Ile Pro Ser Asp Leu													
	30				35				40				
CCG AGG AAT GCC ATT GAA CTG AGG TTT GTC CTC ACC AAG CTT CGA GTC	254												
Pro Arg Asn Ala Ile Glu Leu Arg Phe Val Leu Thr Lys Leu Arg Val													
	45				50				55			60	
ATC CAA AAA GGT GCA TTT TCA GGA TTT GGG GAC CTG GAG AAA ATA GAG	302												
Ile Gln Lys Gly Ala Phe Ser Gly Phe Gly Asp Leu Glu Lys Ile Glu													
		65					70				75		
ATC TCT CAG AAT GAT GTC TTG GAG GTG ATA GAG GCA GAT GTG TTC TCC	350												
Ile Ser Gln Asn Asp Val Leu Glu Val Ile Glu Ala Asp Val Phe Ser													
		80				85				90			
AAC CTT CCC AAA TTA CAT GAA ATT AGA ATT GAA AAG GCC AAC AAC CTG	398												
Asn Leu Pro Lys Leu His Glu Ile Arg Ile Glu Lys Ala Asn Asn Leu													
		95				100				105			
CTC TAC ATC AAC CCT GAG GCC TTC CAG AAC CTT CCC AAC CTT CAA TAT	446												
Leu Tyr Ile Asn Pro Glu Ala Phe Gln Asn Leu Pro Asn Leu Gln Tyr													
	110				115				120				
CTG TTA ATA TCC AAC ACA GGT ATT AAG CAC CTT CCA GAT GTT CAC AAG	494												
Leu Leu Ile Ser Asn Thr Gly Ile Lys His Leu Pro Asp Val His Lys													
	125				130				135			140	
ATT CAT TCT CTC CAA AAA GTT TTA CTT GAC ATT CAA GAT AAC ATA AAC	542												
Ile His Ser Leu Gln Lys Val Leu Leu Asp Ile Gln Asp Asn Ile Asn													
		145					150				155		
ATC CAC ACA ATT GAA AGA AAT TCT TTC GTG GGG CTG AGC TTT GAA AGT	590												
Ile His Thr Ile Glu Arg Asn Ser Phe Val Gly Leu Ser Phe Glu Ser													
		160				165				170			
GTG ATT CTA TGG CTG AAT AAG AAT GGG ATT CAA GAA ATA CAC AAC TGT	638												
Val Ile Leu Trp Leu Asn Lys Asn Gly Ile Gln Glu Ile His Asn Cys													
		175				180				185			
GCA TTC AAT GGA ACC CAA CTA GAT GAG CTG AAT CTA AGC GAT AAT AAT	686												
Ala Phe Asn Gly Thr Gln Leu Asp Glu Leu Asn Leu Ser Asp Asn Asn													
	190				195				200				
AAT TTA GAA GAA TTG CCT AAT GAT GTT TTC CAC GGA GCC TCT GGA CCA	734												
Asn Leu Glu Glu Leu Pro Asn Asp Val Phe His Gly Ala Ser Gly Pro													
	205				210				215			220	
GTC ATT CTA GAT ATT TCA AGA ACA AGG ATC CAT TCC CTG CCT AGC TAT	782												
Val Ile Leu Asp Ile Ser Arg Thr Arg Ile His Ser Leu Pro Ser Tyr													
		225					230				235		
GGC TTA GAA AAT CTT AAG AAG CTG AGG GCC AGG TCG ACT TAC AAC TTA	830												
Gly Leu Glu Asn Leu Lys Lys Leu Arg Ala Arg Ser Thr Tyr Asn Leu													
		240				245					250		

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AAA	AAG	CTG	CCT	ACT	CTG	GAA	AAG	CTT	GTC	GCC	CTC	ATG	GAA	GCC	AGC	878
Lys	Lys	Leu	Pro	Thr	Leu	Glu	Lys	Leu	Val	Ala	Leu	Met	Glu	Ala	Ser	
		255					260					265				
CTC	ACC	TAT	CCC	AGC	CAT	TGC	TGT	GCC	TTT	GCA	AAC	TGG	AGA	CGG	CAA	926
Leu	Thr	Tyr	Pro	Ser	His	Cys	Cys	Ala	Phe	Ala	Asn	Trp	Arg	Arg	Gln	
		270				275					280					
ATC	TCT	GAG	CTT	CAT	CCA	ATT	TGC	AAC	AAA	TCT	ATT	TTA	AGG	CAA	GAA	974
Ile	Ser	Glu	Leu	His	Pro	Ile	Cys	Asn	Lys	Ser	Ile	Leu	Arg	Gln	Glu	
		285			290				295						300	
GTT	GAT	TAT	ATG	ACT	CAG	ACT	AGG	GGT	CAG	AGA	TCC	TCT	CTG	GCA	GAA	1022
Val	Asp	Tyr	Met	Thr	Gln	Thr	Arg	Gly	Gln	Arg	Ser	Ser	Leu	Ala	Glu	
				305					310					315		
GAC	AAT	GAG	TCC	AGC	TAC	AGC	AGA	GGA	TTT	GAC	ATG	ACG	TAC	ACT	GAG	1070
Asp	Asn	Glu	Ser	Ser	Tyr	Ser	Arg	Gly	Phe	Asp	Met	Thr	Tyr	Thr	Glu	
			320					325					330			
TTT	GAC	TAT	GAC	TTA	TGC	AAT	GAA	GTG	GTT	GAC	GTG	ACC	TGC	TCC	CCT	1118
Phe	Asp	Tyr	Asp	Leu	Cys	Asn	Glu	Val	Val	Asp	Val		Cys	Ser	Pro	
		335					340					345				
AAG	CCA	GAT	GCA	TTC	AAC	CCA	TGT	GAA	GAT	ATC	ATG	GGG	TAC	AAC	ATC	1166
Lys	Pro	Asp	Ala	Phe	Asn	Pro	Cys	Glu	Asp	Ile	Met	Gly	Tyr	Asn	Ile	
		350				355					360					
CTC	AGA	GTC	CTG	ATA	TGG	TTT	ATC	AGC	ATC	CTG	GCC	ATC	ACT	GGG	AAC	1214
Leu	Arg	Val	Leu	Ile	Trp	Phe	Ile	Ser	Ile	Leu	Ala	Ile	Thr	Gly	Asn	
		365			370					375					380	
ATC	ATA	GTG	CTA	GTG	ATC	CTA	ACT	ACC	AGC	CAA	TAT	AAA	CTC	ACA	GTC	1262
Ile	Ile	Val	Leu	Val	Ile	Leu	Thr	Thr	Ser	Gln	Tyr	Lys	Leu	Thr	Val	
				385					390					395		
CCC	AGG	TTC	CTT	ATG	TGC	AAC	CTG	GCC	TTT	GCT	GAT	CTC	TGC	ATT	GGA	1310
Pro	Arg	Phe	Leu	Met	Cys	Asn	Leu	Ala	Phe	Ala	Asp	Leu	Cys	Ile	Gly	
			400					405					410			
ATC	TAC	CTG	CTG	CTC	ATT	GCA	TCA	GTT	GAT	ATC	CAT	ACC	AAG	AGC	CAA	1358
Ile	Tyr	Leu	Leu	Leu	Ile	Ala	Ser	Val	Asp	Ile	His	Thr	Lys	Ser	Gln	
		415					420					425				
TAT	CAC	AAC	TAT	GCC	ATT	GAC	TGG	CAA	ACT	GGG	GCA	GGC	TGT	GAT	GCT	1406
Tyr	His	Asn	Tyr	Ala	Ile	Asp	Trp	Gln	Thr	Gly	Ala	Gly	Cys	Asp	Ala	
		430				435					440					
GCT	GGC	TTT	TTC	ACT	GTC	TTT	GCC	AGT	GAG	CTG	TCA	GTC	TAC	ACT	CTG	1454
Ala	Gly	Phe	Phe	Thr	Val	Phe	Ala	Ser	Glu	Leu	Ser	Val	Tyr	Thr	Leu	
		445			450					455					460	
ACA	GCT	ATC	ACC	TTG	GAA	AGA	TGG	CAT	ACC	ATC	ACG	CAT	GCC	ATG	CAG	1502
Thr	Ala	Ile	Thr	Leu	Glu	Arg	Trp	His	Thr	Ile	Thr	His	Ala	Met	Gln	
				465				470						475		
CTG	GAC	TGC	AAG	GTG	CAG	CTC	CGC	CAT	GCT	GCC	AGT	GTC	ATG	GTG	ATG	1550
Leu	Asp	Cys	Lys	Val	Gln	Leu	Arg	His	Ala	Ala	Ser	Val	Met	Val	Met	
			480					485					490			
GGC	TGG	ATT	TTT	GCT	TTT	GCA	GCT	GCC	CTC	TTT	CCC	ATC	TTT	GGC	ATC	1598
Gly	Trp	Ile	Phe	Ala	Phe	Ala	Ala	Ala	Leu	Phe	Pro	Ile	Phe	Gly	Ile	
		495					500					505				

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AGC	AGC	TAC	ATG	AAG	GTG	AGC	ATC	TGC	CTG	CCC	ATG	GAT	ATT	GAC	AGC	1646
Ser	Ser	Tyr	Met	Lys	Val	Ser	Ile	Cys	Leu	Pro	Met	Asp	Ile	Asp	Ser	
510						515					520					
CCT	TTG	TCA	CAG	CTG	TAT	GTC	ATG	TCC	CTC	CTT	GTG	CTC	AAT	GTC	CTG	1694
Pro	Leu	Ser	Gln	Leu	Tyr	Val	Met	Ser	Leu	Leu	Val	Leu	Asn	Val	Leu	
525					530					535					540	
GCC	TTT	GTG	GTC	ATC	TGT	GGC	TGC	TAT	ATC	CAC	ATC	TAC	CTC	ACA	GTG	1742
Ala	Phe	Val	Val	Ile	Cys	Gly	Cys	Tyr	Ile	His	Ile	Tyr	Leu	Thr	Val	
				545					550					555		
CGG	AAC	CCC	AAC	ATC	GTG	TCC	TCC	TCT	AGT	GAC	ACC	AGG	ATC	GCC	AAG	1790
Arg	Asn	Pro	Asn	Ile	Val	Ser	Ser	Ser	Ser	Asp	Thr	Arg	Ile	Ala	Lys	
			560					565					570			
CGC	ATG	GCC	ATG	CTC	ATC	TTC	ACT	GAC	TTC	CTC	TGC	ATG	GCA	CCC	ATT	1838
Arg	Met	Ala	Met	Leu	Ile	Phe	Thr	Asp	Phe	Leu	Cys	Met	Ala	Pro	Ile	
	575					580					585					
TCT	TTC	TTT	GCC	ATT	TCT	GCC	TCC	CTC	AAG	GTG	CCC	CTC	ATC	ACT	GTG	1886
Ser	Phe	Phe	Ala	Ile	Ser	Ala	Ser	Leu	Lys	Val	Pro	Leu	Ile	Thr	Val	
	590					595					600					
TCC	AAA	GCA	AAG	ATT	CTG	CTG	GTT	CTG	TTT	CAC	CCC	ATC	AAC	TCC	TGT	1934
Ser	Lys	Ala	Lys	Ile	Leu	Leu	Val	Leu	Phe	His	Pro	Ile	Asn	Ser	Cys	
605					610					615					620	
GCC	AAC	CCC	TTC	CTC	TAT	GCC	ATC	TTT	ACC	AAA	AAC	TTT	CGC	AGA	GAT	1982
Ala	Asn	Pro	Phe	Leu	Tyr	Ala	Ile	Phe	Thr	Lys	Asn	Phe	Arg	Arg	Asp	
			625					630						635		
TTC	TTC	ATT	CTG	CTG	AGC	AAG	TGT	GGC	TGC	TAT	GAA	ATG	CAA	GCC	CAA	2030
Phe	Phe	Ile	Leu	Leu	Ser	Lys	Cys	Gly	Cys	Tyr	Glu	Met	Gln	Ala	Gln	
			640					645					650			
ATT	TAT	AGG	ACA	GAA	ACT	TCA	TCC	ACT	GTC	CAC	AAC	ACC	CAT	CCA	AGG	2078
Ile	Tyr	Arg	Thr	Glu	Thr	Ser	Ser	Thr	Val	His	Asn	Thr	His	Pro	Arg	
		655					660					665				
AAT	GGC	CAC	TGC	TCT	TCA	GCT	CCC	AGA	GTC	ACC	AAT	GGT	TCC	ACT	TAC	2126
Asn	Gly	His	Cys	Ser	Ser	Ala	Pro	Arg	Val	Thr	Asn	Gly	Ser	Thr	Tyr	
	670					675					680					
ATA	CTT	GTC	CCT	CTA	AGT	CAT	TTA	GCC	CAA	AAC	TAAACACAA	TGTGAAAATG				2179
Ile	Leu	Val	Pro	Leu	Ser	His	Leu	Ala	Gln	Asn						
685					690					695						

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 62 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGCCCGCCGC	GCCCGCGCC	CGGCCCGCCG	CCCCGCCCCG	GACTTATGCA	ATGAAGTGGT	60
TG						62

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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTGAAAAAGC CAGCAGCATC

20

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 59 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGCCCCCGCGC GCCCGCGGCC CGGCCCCGCG CCCCGCCCCG ATTGACTGGC AAAGTGGGG

59

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGAGGAGGAC ACGATGTTGG

20

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 62 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGCCCCCGCGC GCCCGCGGCC CGGCCCCGCG CCCCGCCCCG GGCTGCTATA TCCACATCTA

60

CC

62

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- 23 -

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGAACCAGC AGAATCTTTG C

21

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 60 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGCCCGCCGC GCCCGCGGCC CGGCCCGCCG CCCCCGCCCG CTTTCTTTGC CATTTCTGCC

60

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CAAAGGCAAG GACTGAATTA TC

22

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGCAAAGATT CTGCTGGTTC

20

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TAGTTTTGGG CTAAATGACT TAGAGGG

27

- 24 -

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCTGCTCCTG GTCTCTTTGC TG

22

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGAAATTCTT TCGTGGGGCT

20

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTTTGCAAAG GCACAGCAAT

20

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTTATTTCAG ATGGCTGAAT AAG

23

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCTCATCTAG TTGGGTTC

18

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GAGTGCACCA TGGGCGGTGT GAAAT

25

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGGATTCAAG AAATACACAA CTGTGTATTC AATGGAACCC

40

CLAIMS

We claim:

1. A method for determining a follicle-stimulating hormone receptor (*fshr*) genotype in a human patient, comprising the steps of:
 - 5 (a) providing a biological sample comprising nucleic acid from said patient, said nucleic acid including said patient's *fshr* alleles;
 - (b) analyzing said nucleic acid for the presence of a mutation or mutations in codon 189 of said *fshr* alleles; and
 - (c) determining an *fshr* genotype from said analyzing step, where-
10 rein the presence of a mutation in codon 189 of a *fshr* allele is correlated with an ovarian dysgenesis genotype.
2. The method according to claim 1 wherein said biological sample is a cell sample.
3. The method according to claim 1 or 2 wherein said patient is a
15 female.
4. The method according to any one of claims 1-3 wherein said analyzing comprises sequencing a portion of said nucleic acid, said portion comprising codon 189 of said *fshr* alleles.
5. The method according to any one of claims 1-4 wherein said nucleic
20 acid is DNA.
6. The method according to claim 5 wherein said analyzing step comprises the steps of:
 - (a) exposing said nucleic acid to a restriction endonuclease having a recognition site that includes codon 189 of a wild type *fshr* allele, under
25 conditions wherein said restriction endonuclease specifically cleaves DNA at its recognition site; and

(b) detecting said nucleic acid or polynucleotide restriction fragments thereof from said exposing step.

7. The method according to claim 6 wherein said restriction endonuclease is selected from the group consisting of *BsmI*, restriction endonucleases
5 recognizing sites that overlap a *BsmI* site, restriction endonucleases recognizing the same cleavage sites as *BsmI*, and isoschizomers of *BsmI*.

8. The method according to claim 6 wherein said restriction endonuclease is *BsmI*.

9. A method for screening for an ovarian dysgenesis genotype in a
10 patient, comprising the steps of:

(a) providing a biological sample comprising nucleic acid from said patient, said nucleic acid including said patient's follicle-stimulating hormone receptor (*fshr*) alleles;

(b) amplifying a portion of said nucleic acid to generate amplified
15 DNA, said portion including codon 189 of said *fshr* alleles;

(c) exposing said amplified DNA to a restriction endonuclease selected from the group consisting of *BsmI* restriction endonuclease and restriction endonucleases having a recognition site which overlaps a *BsmI* recognition site, under conditions wherein said restriction endonuclease specifically cleaves DNA at
20 the recognition site of said restriction endonuclease;

(d) thereafter detecting said amplified DNA or restriction fragments thereof;

(e) comparing the amplified DNA or fragments thereof of step (d) to control nucleic acid of a human subject free of an ovarian dysgenesis genotype, wherein said control nucleic acid has been amplified, exposed, and detected in
25 accordance with steps (b), (c), and (d); and

(f) screening for an ovarian dysgenesis genotype from said comparison, wherein an ovarian dysgenesis genotype in said patient is correlated with a

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different number of detected amplified DNA or restriction fragments thereof from said patient's amplified DNA than from said control amplified DNA.

10. The method according to claim 9 wherein in said screening step a homozygous ovarian dysgenesis genotype is correlated with fewer detected restriction fragments from said patient's amplified DNA than from said control amplified DNA.

11. The method according to claim 9 wherein in said screening step a heterozygous ovarian dysgenesis genotype is correlated with a greater number of detected amplified DNA or restriction fragments thereof from said patient's amplified DNA than from said control amplified DNA.

12. The method according to claim 9 wherein said patient is a human female.

13. The method according to claim 9 wherein said portion comprises exon 7 of said *fshr* alleles.

14. A method for diagnosing ovarian dysgenesis in a female patient, comprising the steps of:

obtaining a cell sample from a female patient;

isolating nucleic acids from said cell sample;

amplifying a portion of said nucleic acids encoding a receptor for follicle-stimulating hormone, thereby generating amplified DNA;

exposing said amplified DNA to a restriction endonuclease selected from the group consisting of *BsmI* and restriction endonucleases having a recognition site which overlaps that of *BsmI*, under conditions wherein said restriction endonuclease specifically cleaves DNA at its recognition site;

detecting polynucleotide restriction fragments of said amplified DNA; and

diagnosing ovarian dysgenesis from said restriction fragments, wherein ovarian dysgenesis is correlated with a C to T mutation in codon 189 of said DNA encoding a receptor for follicle-stimulating hormone, said mutation eliminating a recognition site of said restriction endonuclease.

- 5 15. A method for diagnosing ovarian dysgenesis in a female patient comprising the steps of:
- obtaining a cell sample from a female patient;
 - isolating nucleic acids from said cell sample;
 - amplifying a portion of said nucleic acids comprising exon 7 of a
 - 10 follicle-stimulating hormone receptor gene, said portion excluding any *BsmI* site outside of exon 7, thereby generating amplified DNA;
 - exposing said amplified DNA to a restriction endonuclease selected from the group consisting of *BsmI* and restriction endonucleases having a recognition site which overlaps that of *BsmI*, under conditions wherein said restriction
 - 15 endonuclease specifically cleaves DNA at its recognition site;
 - detecting said amplified DNA or polynucleotide restriction fragments thereof after said exposing step; and
 - diagnosing ovarian dysgenesis from said detecting step, wherein ovarian dysgenesis is correlated with the detection of said amplified DNA and the
 - 20 absence of polynucleotide restriction fragments thereof.

16. The method according to claim 14 or 15, wherein said restriction endonucleases having a recognition site which overlaps that of *BsmI* are selected from the group consisting of *SacIII* and *SstIII*.

17. A method for diagnosing ovarian dysgenesis in a female patient
- 25 suspected of having ovarian dysgenesis, comprising the steps of:
- obtaining a cell sample from said female patient;
 - isolating nucleic acid from said cell sample;

- 30 -

amplifying a portion of said isolated nucleic acid comprising exon 7
of a follicle-stimulating hormone receptor gene, thereby generating amplified DNA;
sequencing said amplified DNA;
comparing the sequence of said amplified DNA to SEQ ID NO:1;

5 and

diagnosing ovarian dysgenesis by the presence of one or more se-
quence differences between the sequence of said amplified DNA and SEQ ID
NO:1.

10 18. The method according to claim 17, wherein said one or more diffe-
rences between the sequence of said amplified DNA and SEQ ID NO:1 comprises
a difference at nucleotide position 640 in SEQ ID NO:1.

19. The method according to claim 18, wherein a cytidylate at position
640 in SEQ ID NO:1 is a thymidylate at a corresponding position in said amplified
DNA.

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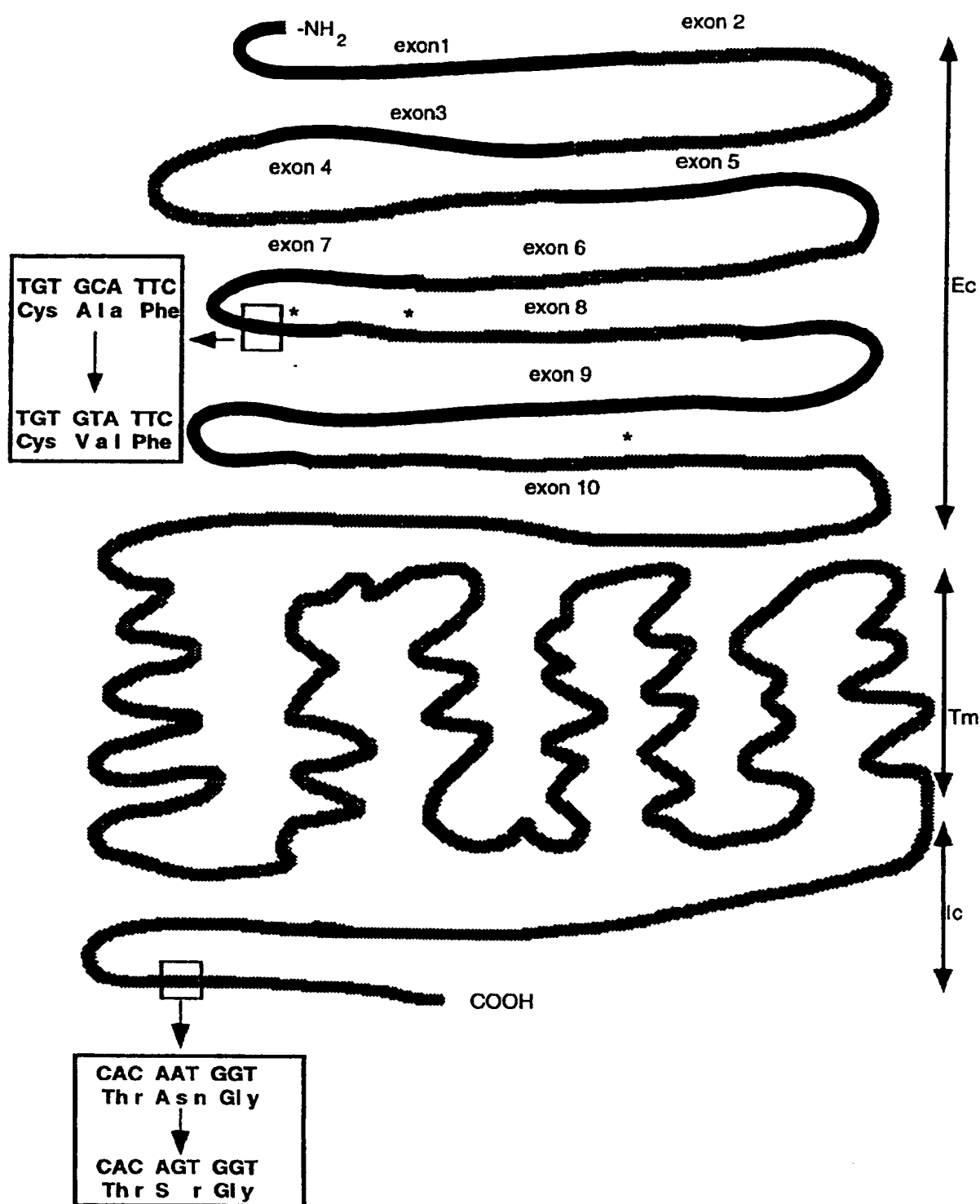


FIG. 1

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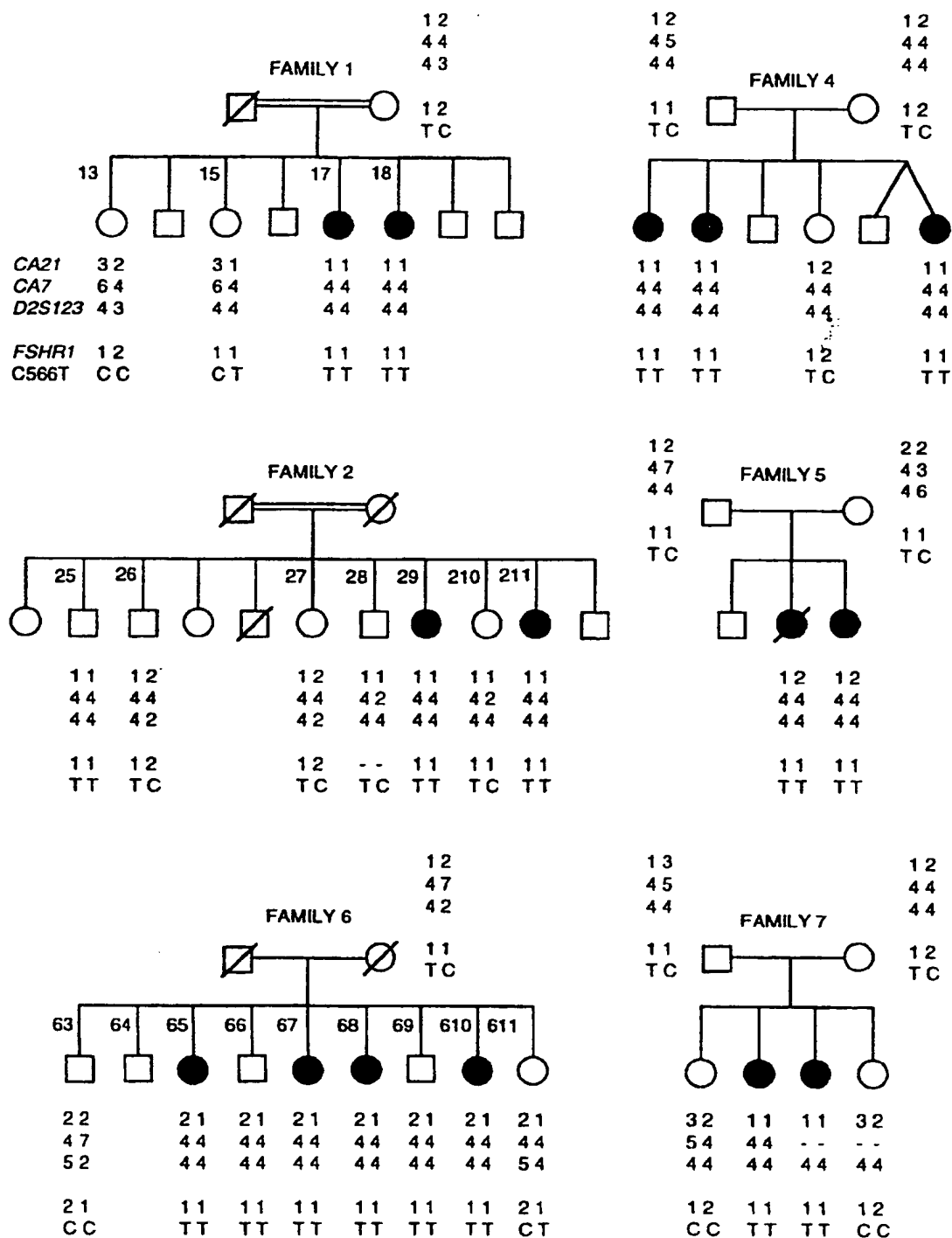
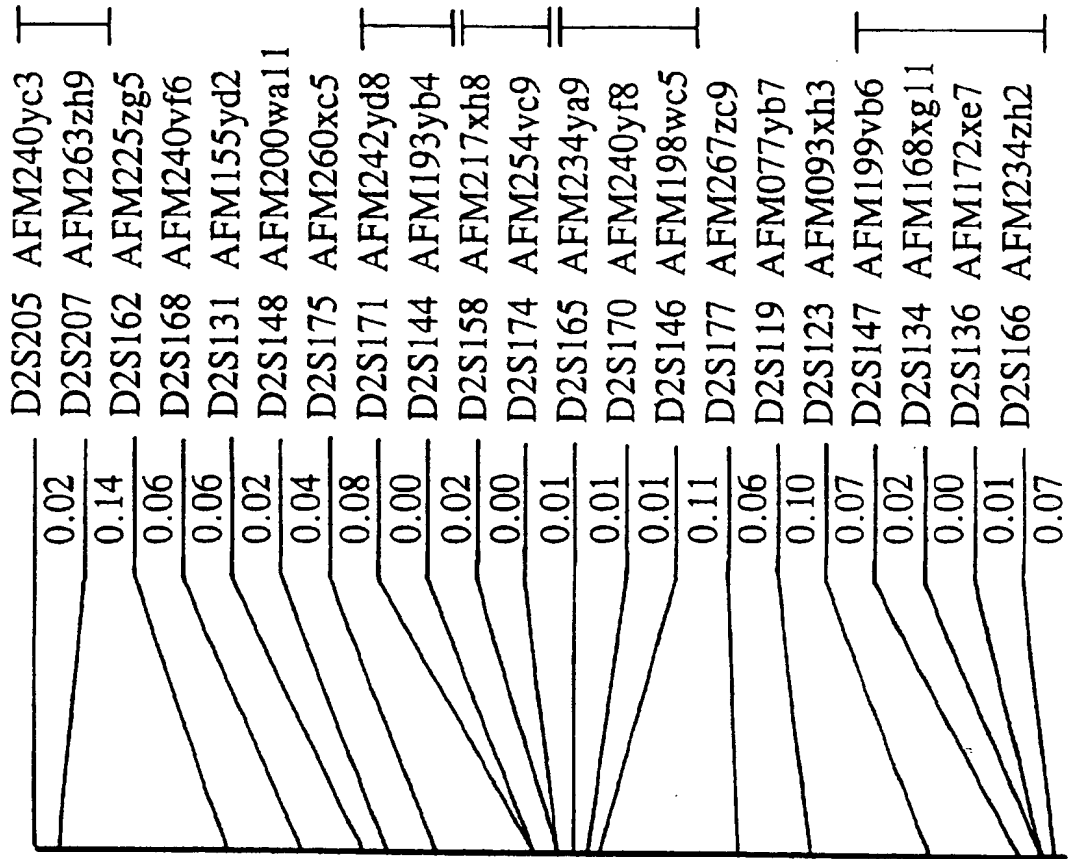


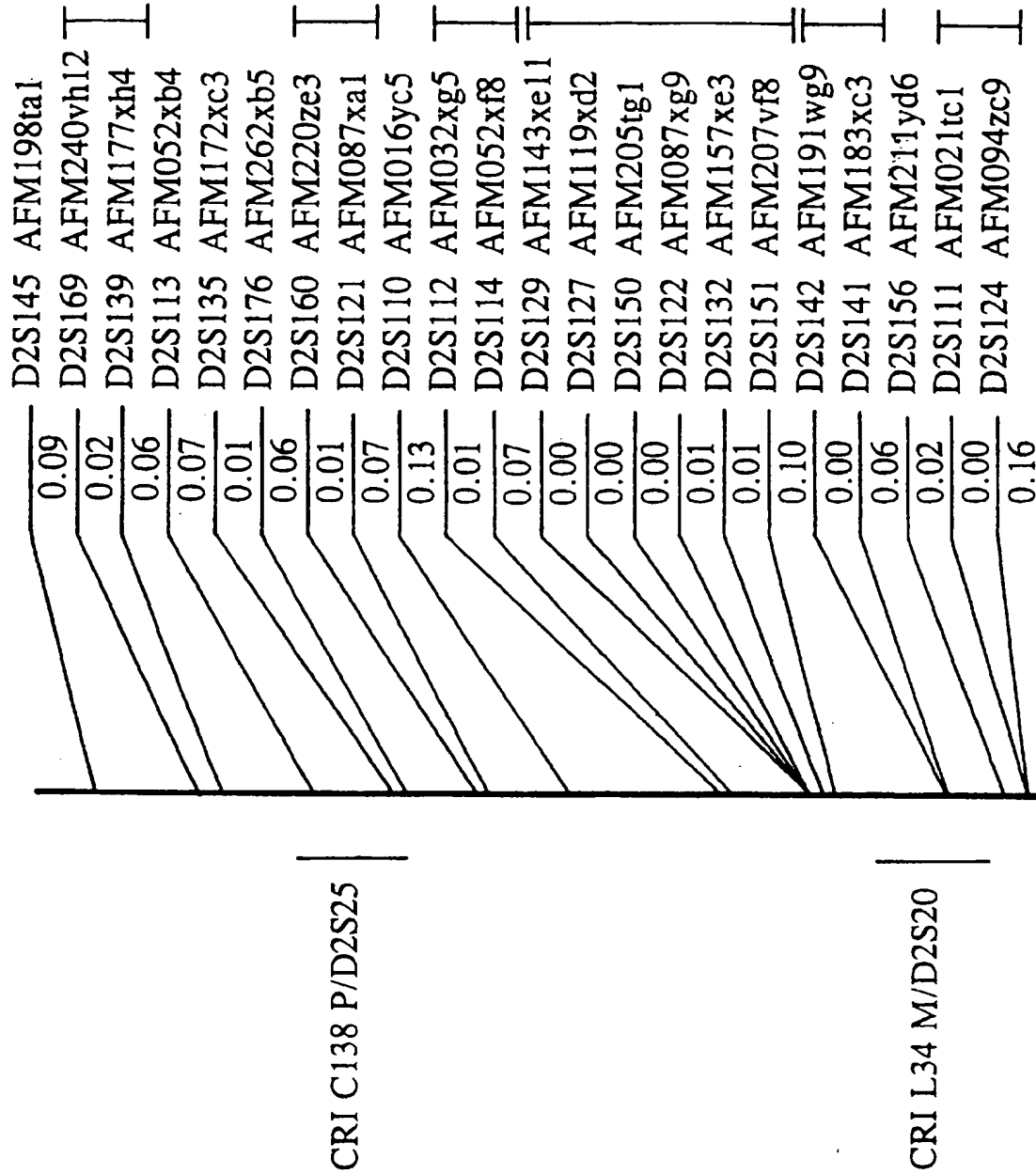
FIG. 2

pTBAB 5-7 Py/D2S47

E28-T/D2S16

CRI L1247 M/D2S34





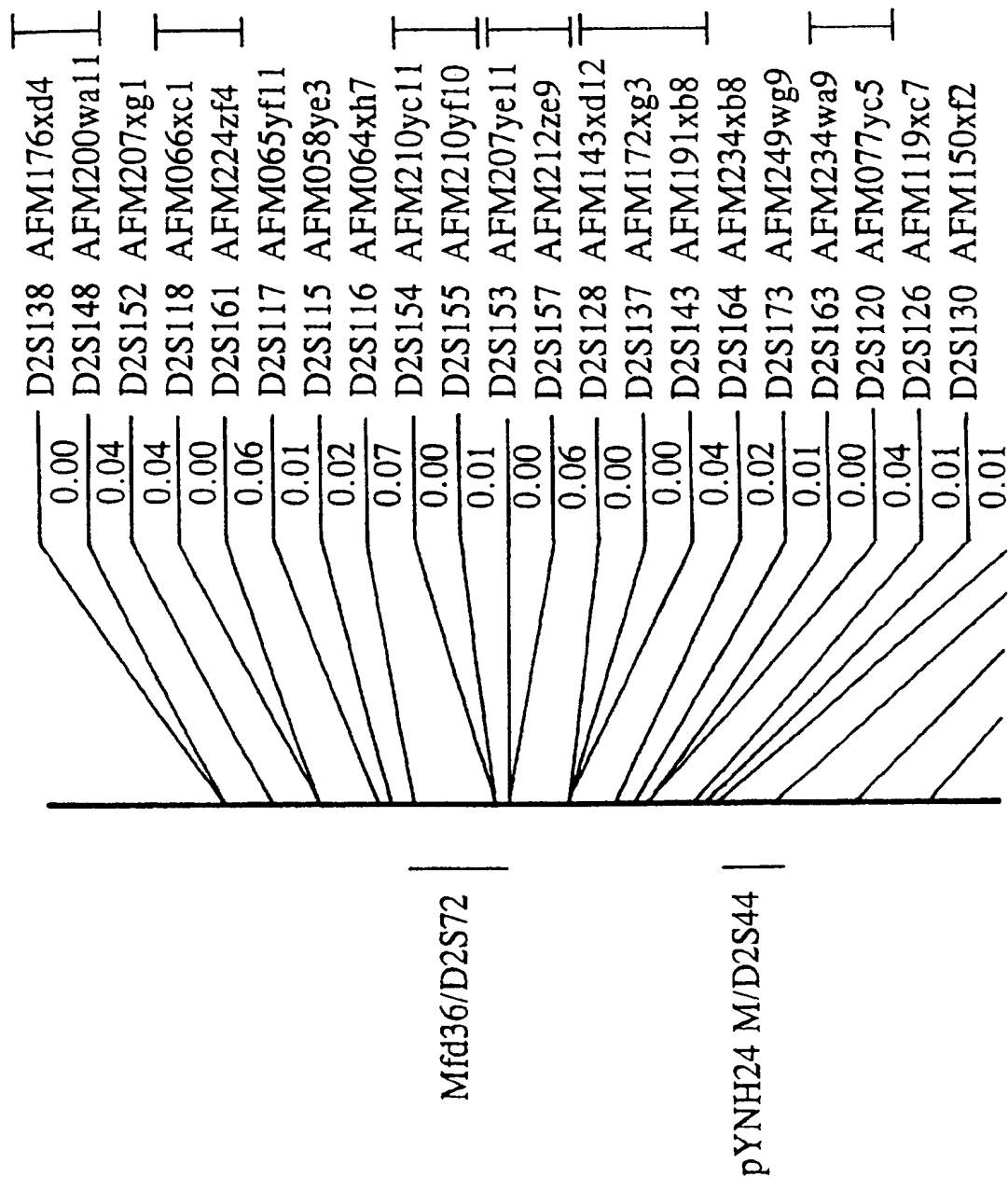


FIG. 3 (3of 4)

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FIG. 3 (4 of 4)

	D2S133	AFM165zh8
0.06	D2S159	AFM218zg3
0.07	D2S172	AFM248wc5
0.06	D2S206	AFM259yc9
0.22	D2S125	AFM112yd4
0.04	D2S140	AFM182ya5

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TGTGGAGCTT	CTGAGATCTG	TGGAGGTTTT	TCTCTGCAAA	TGCAGGAAGA	AATCAGGTGG		60									
ATGGATGCAT	AATT	ATG	GCC	CTG	CTC	CTG	GTC	TCT	TTG	CTG	GCA	TTC	CTG		110	
		Met	Ala	Leu	Leu	Leu	Val	Ser	Leu	Leu	Ala	Phe	Leu		12	
AGC	TTG	GGC	TCA	GGA	TGT	CAT	CAT	CGG	ATC	TGT	CAC	TGC	TCT	AAC	AGG	158
Ser	Leu	Gly	Ser	Gly	Cys	His	His	Arg	Ile	Cys	His	Cys	Ser	Asn	Arg	28
GTT	TTT	CTC	TGC	CAA	GAG	AGC	AAG	GTG	ACA	GAG	ATT	CCT	TCT	GAC	CTC	206
Val	Phe	Leu	Cys	Gln	Glu	Ser	Lys	Val	Thr	Glu	Ile	Pro	Ser	Asp	Leu	44
CCG	AGG	AAT	GCC	ATT	GAA	CTG	AGG	TTT	GTC	CTC	ACC	AAG	CTT	CGA	GTC	254
Pro	Arg	Asn	Ala	Ile	Glu	Leu	Arg	Phe	Val	Leu	Thr	Lys	Leu	Arg	Val	60
ATC	CAA	AAA	GGT	GCA	TTT	TCA	GGA	TTT	GGG	GAC	CTG	GAG	AAA	ATA	GAG	302
Ile	Gln	Lys	Gly	Ala	Phe	Ser	Gly	Phe	Gly	Asp	Leu	Glu	Lys	Ile	Glu	76
ATC	TCT	CAG	AAT	GAT	GTC	TTG	GAG	GTG	ATA	GAG	GCA	GAT	GTG	TTC	TCC	350
Ile	Ser	Gln	Asn	Asp	Val	Leu	Glu	Val	Ile	Glu	Ala	Asp	Val	Phe	Ser	92
AAC	CTT	CCC	AAA	TTA	CAT	GAA	ATT	AGA	ATT	GAA	AAG	GCC	AAC	AAC	CTG	398
Asn	Leu	Pro	Lys	Leu	His	Glu	Ile	Arg	Ile	Glu	Lys	Ala	Asn	Asn	Leu	108
CTC	TAC	ATC	AAC	CCT	GAG	GCC	TTC	CAG	AAC	CTT	CCC	AAC	CTT	CAA	TAT	446
Leu	Tyr	Ile	Asn	Pro	Glu	Ala	Phe	Gln	Asn	Leu	Pro	Asn	Leu	Gln	Tyr	124
CTG	TTA	ATA	TCC	AAC	ACA	GGT	ATT	AAG	CAC	CTT	CCA	GAT	GTT	CAC	AAG	494
Leu	Leu	Ile	Ser	Asn	Thr	Gly	Ile	Lys	His	Leu	Pro	Asp	Val	His	Lys	140
ATT	CAT	TCT	CTC	CAA	AAA	GTT	TTA	CTT	GAC	ATT	CAA	GAT	AAC	ATA	AAC	542
Ile	His	Ser	Leu	Gln	Lys	Val	Leu	Leu	Asp	Ile	Gln	Asp	Asn	Ile	Asn	156
ATC	CAC	ACA	ATT	GAA	AGA	AAT	TCT	TTC	GTG	GGG	CTG	AGC	TTT	GAA	AGT	590
Ile	His	Thr	Ile	Glu	Arg	Asn	Ser	Phe	Val	Gly	Leu	Ser	Phe	Glu	Ser	172
GTG	ATT	CTA	TGG	CTG	AAT	AAG	AAT	GGG	ATT	CAA	GAA	ATA	CAC	AAC	TGT	638
Val	Ile	Leu	Trp	Leu	Asn	Lys	Asn	Gly	Ile	Gln	Glu	Ile	His	Asn	Cys	188
GCA	TTC	AAT	GGA	ACC	CAA	CTA	GAT	GAG	CTG	AAT	CTA	AGC	GAT	AAT	AAT	686
Ala	Phe	Asn	Gly	Thr	Gln	Leu	Asp	Glu	Leu	Asn	Leu	Ser	Asp	Asn	Asn	204
AAT	TTA	GAA	GAA	TTG	CCT	AAT	GAT	GTT	TTC	CAC	GGA	GCC	TCT	GGA	CCA	734
Asn	Leu	Glu	Glu	Leu	Pro	Asn	Asp	Val	Phe	His	Gly	Ala	Ser	Gly	Pro	220
GTC	ATT	CTA	GAT	ATT	TCA	AGA	ACA	AGG	ATC	CAT	TCC	CTG	CCT	AGC	TAT	782
Val	Ile	Leu	Asp	Ile	Ser	Arg	Thr	Arg	Ile	His	Ser	Leu	Pro	Ser	Tyr	236
GGC	TTA	GAA	AAT	CTT	AAG	AAG	CTG	AGG	GCC	AGG	TCG	ACT	TAC	AAC	TTA	830
Gly	Leu	Glu	Asn	Leu	Lys	Lys	Leu	Arg	Ala	Arg	Ser	Thr	Tyr	Asn	Leu	252
AAA	AAG	CTG	CCT	ACT	CTG	GAA	AAG	CTT	GTC	GCC	CTC	ATG	GAA	GCC	AGC	878
Lys	Lys	Leu	Pro	Thr	Leu	Glu	Lys	Leu	Val	Ala	Leu	Met	Glu	Ala	Ser	268
CTC	ACC	TAT	CCC	AGC	CAT	TGC	TGT	GCC	TTT	GCA	AAC	TGG	AGA	CGG	CAA	926
Leu	Thr	Tyr	Pro	Ser	His	Cys	Cys	Ala	Phe	Ala	Asn	Trp	Arg	Arg	Gln	284
ATC	TCT	GAG	CTT	CAT	CCA	ATT	TGC	AAC	AAA	TCT	ATT	TTA	AGG	CAA	GAA	974
Ile	Ser	Glu	Leu	His	Pro	Ile	Cys	Asn	Lys	Ser	Ile	Leu	Arg	Gln	Glu	300
GTT	GAT	TAT	ATG	ACT	CAG	ACT	AGG	GGT	CAG	AGA	TCC	TCT	CTG	GCA	GAA	1022
Val	Asp	Tyr	Met	Thr	Gln	Thr	Arg	Gly	Gln	Arg	Ser	Ser	Leu	Ala	Glu	316
GAC	AAT	GAG	TCC	AGC	TAC	AGC	AGA	GGA	TTT	GAC	ATG	ACG	TAC	ACT	GAG	1070
Asp	Asn	Glu	Ser	Ser	Tyr	Ser	Arg	Gly	Phe	Asp	Met	Thr	Tyr	Thr	Glu	332
TTT	GAC	TAT	GAC	TTA	TGC	AAT	GAA	GTG	GTT	GAC	GTG	ACC	TGC	TCC	CCT	1118
Phe	Asp	Tyr	Asp	Leu	Cys	Asn	Glu	Val	Val	Asp	Val	Thr	Cys	Ser	Pro	348

FIG. 4 (1 of 2)

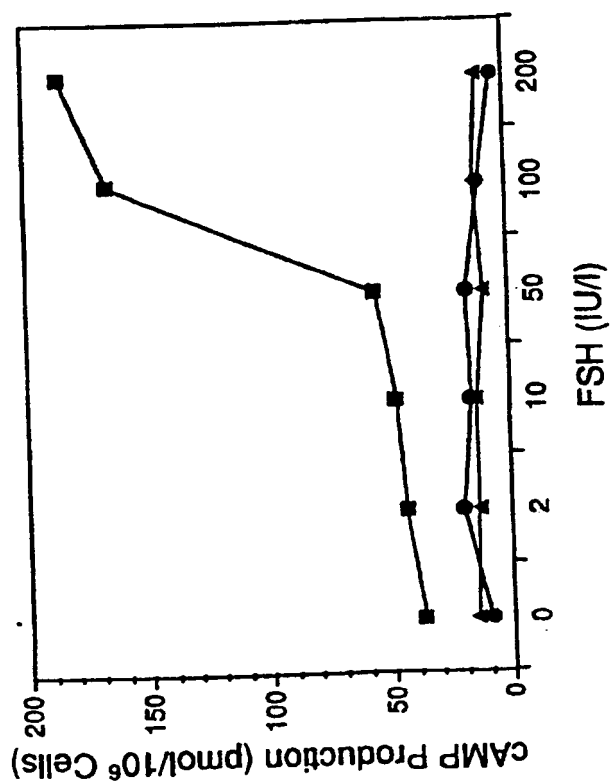
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AAG Lys	CCA Pro	GAT Asp	GCA Ala	TTC Phe	AAC Asn	CCA Pro	TGT Cys	GAA Glu	GAT Asp	ATC Ile	ATG Met	GGG Gly	TAC Tyr	AAC Asn	ATC Ile	1166 364
CTC Leu	AGA Arg	GTC Val	CTG Leu	ATA Ile	TGG Trp	TTT Phe	ATC Ile	AGC Ser	ATC Ile	CTG Leu	GCC Ala	ATC Ile	ACT Thr	GGG Gly	AAC Asn	1214 380
ATC Ile	ATA Ile	GTG Val	CTA Leu	GTG Val	ATC Ile	CTA Leu	ACT Thr	ACC Thr	AGC Ser	CAA Gln	TAT Tyr	AAA Lys	CTC Leu	ACA Thr	GTC Val	1262 396
CCC Pro	AGG Arg	TTC Phe	CTT Leu	ATG Met	TGC Cys	AAC Asn	CTG Leu	GCC Ala	TTT Phe	GCT Ala	GAT Asp	CTC Leu	TGC Cys	ATT Ile	GGA Gly	1310 412
ATC Ile	TAC Tyr	CTG Leu	CTG Leu	CTC Leu	ATT Ile	GCA Ala	TCA Ser	GTT Val	GAT Asp	ATC Ile	CAT His	ACC Thr	AAG Lys	AGC Ser	CAA Gln	1358 428
TAT Tyr	CAC His	AAC Asn	TAT Tyr	GCC Ala	ATT Ile	GAC Asp	TGG Trp	CAA Gln	ACT Thr	GGG Gly	GCA Ala	GGC Gly	TGT Cys	GAT Asp	GCT Ala	1406 444
GCT Ala	GGC Gly	TTT Phe	TTC Phe	ACT Thr	GTC Val	TTT Phe	GCC Ala	AGT Ser	GAG Glu	CTG Leu	TCA Ser	GTC Val	TAC Tyr	ACT Thr	CTG Leu	1454 460
ACA Thr	GCT Ala	ATC Ile	ACC Thr	TTG Leu	GAA Glu	AGA Arg	TGG Trp	CAT His	ACC Thr	ATC Ile	ACG Thr	CAT His	GCC Ala	ATG Met	CAG Gln	1502 476
CTG Leu	GAC Asp	TGC Cys	AAG Lys	GTG Val	CAG Gln	CTC Leu	CGC Arg	CAT His	GCT Ala	GCC Ala	AGT Ser	GTC Val	ATG Met	GTG Val	ATG Met	1550 492
GGC Gly	TGG Trp	ATT Ile	TTT Phe	GCT Ala	TTT Phe	GCA Ala	GCT Ala	GCC Ala	CTC Leu	TTT Phe	CCC Pro	ATC Ile	TTT Phe	GGC Gly	ATC Ile	1598 508
AGC Ser	AGC Ser	TAC Tyr	ATG Met	AAG Lys	GTG Val	AGC Ser	ATC Ile	TGC Cys	CTG Leu	CCC Pro	ATG Met	GAT Asp	ATT Ile	GAC Asp	AGC Ser	1646 524
CCT Pro	TTG Leu	TCA Ser	CAG Gln	CTG Leu	TAT Tyr	GTC Val	ATG Met	TCC Ser	CTC Leu	CTT Leu	GTG Val	CTC Leu	AAT Asn	GTC Val	CTG Leu	1694 540
GCC Ala	TTT Phe	GTG Val	GTC Val	ATC Ile	TGT Cys	GGC Gly	TGC Cys	TAT Tyr	ATC Ile	CAC His	ATC Ile	TAC Tyr	CTC Leu	ACA Thr	GTG Val	1742 556
CGG Arg	AAC Asn	CCC Pro	AAC Asn	ATC Ile	GTG Val	TCC Ser	TCC Ser	TCT Ser	AGT Ser	GAC Asp	ACC Thr	AGG Arg	ATC Ile	GCC Ala	AAG Lys	1790 572
CGC Arg	ATG Met	GCC Ala	ATG Met	CTC Leu	ATC Ile	TTC Phe	ACT Thr	GAC Asp	TTC Phe	CTC Leu	TGC Cys	ATG Met	GCA Ala	CCC Pro	ATT Ile	1838 588
TCT Ser	TTC Phe	TTT Phe	GCC Ala	ATT Ile	TCT Ser	GCC Ala	TCC Ser	CTC Leu	AAG Lys	GTG Val	CCC Pro	CTC Leu	ATC Ile	ACT Thr	GTG Val	1886 604
TCC Ser	AAA Lys	GCA Ala	AAG Lys	ATT Ile	CTG Leu	CTG Leu	GTT Val	CTG Leu	TTT Phe	CAC His	CCC Pro	ATC Ile	AAC Asn	TCC Ser	TGT Cys	1934 620
GCC Ala	AAC Asn	CCC Pro	TTC Phe	CTC Leu	TAT Tyr	GCC Ala	ATC Ile	TTT Phe	ACC Thr	AAA Lys	AAC Asn	TTT Phe	CGC Arg	AGA Arg	GAT Asp	1982 636
TTC Phe	TTC Phe	ATT Ile	CTG Leu	CTG Leu	AGC Ser	AAG Lys	TGT Cys	GGC Gly	TGC Cys	TAT Tyr	GAA Glu	ATG Met	CAA Gln	GCC Ala	CAA Gln	2030 652
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AAT Asn	GGC Gly	CAC His	TGC Cys	TCT Ser	TCA Ser	GCT Ala	CCC Pro	AGA Arg	GTC Val	ACC Thr	AAT Asn	GGT Gly	TCC Ser	ACT Thr	TAC Tyr	2126 684
ATA Ile	CTT Leu	GTC Val	CCT Pro	CTA Leu	AGT Ser	CAT His	TTA Leu	GCC Ala	CAA Gln	AAC Asn	TAAAACACAA				TGTGAAAATG	2179 695

FIG. 4 (2 of 2)

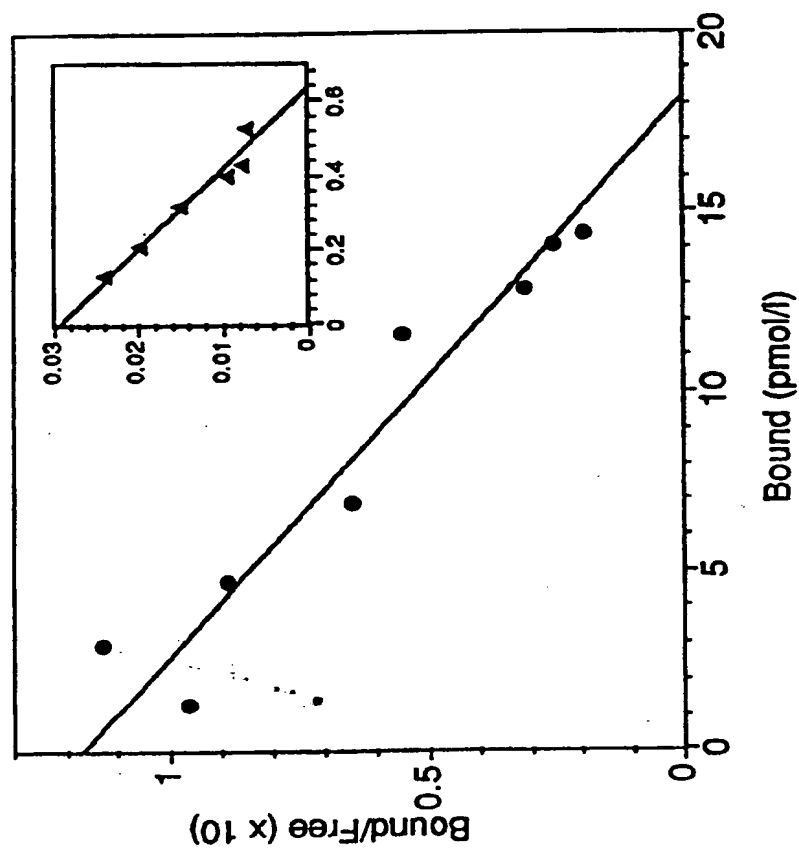
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FIG. 5



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FIG. 6



INTERNATIONAL SEARCH REPORT

International Application No
PCT/FI 96/00501

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 C07H21/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	CELL, vol. 82, no. 6, 22 September 1995, pages 959-68, XP000609143 AITTOMÄKI, K. ET AL: "Mutation in the follicle-stimulating hormone receptor causes hereditary hypergonadotropic ovarian failure" see the whole document ---	1-18
A	AMERICAN JOURNAL OF HUMAN GENETICS, vol. 54, no. 5, May 1994, pages 844-51, XP000612836 AITTOMÄKI, K.: "The genetics of XX gonadal dysgenesis" cited in the application see the whole document --- -/-	1

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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Date of the actual completion of the international search

11 December 1996

Date of mailing of the international search report

07. 01. 97

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Authorized officer

Osborne, H

INTERNATIONAL SEARCH REPORT

Inter- national Application No
PC1/FI 96/00501

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NATURE GENETICS, vol. 9, February 1995, pages 160-4, XP000612866 KREMER H ET AL: "Male pseudohermaphroditism due to a homzygous missense mutation of the luteinizing hormone receptor gene" see the whole document ---</p>	1
A	<p>GENOMICS , vol. 15, January 1993, pages 222-24, XP000612862 ROUSSEAU-MERCK M ET AL: "The chromosomal localization of the human follicle hormone receptor gene (FSHR) on 2p21-2p15 is similar to that of the luteinizing hormone receptor gene" cited in the application see the whole document -----</p>	1

